Transcription factor TnrA inhibits the biosynthetic activity of glutamine synthetase in *Bacillus subtilis*

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A R T I C L E   I N F O

Article history:
Received 25 November 2012
Revised 11 March 2013
Accepted 11 March 2013
Available online 25 March 2013
Edited by Stuart Ferguson

Keywords:
Glutamine synthetase
Nitrogen regulation
Transcription factor TnrA
*Bacillus subtilis*

A B S T R A C T

The *Bacillus subtilis* glutamine synthetase (GS) plays a dual role in cell metabolism by functioning as catalyst and regulator. GS catalyses the ATP-dependent synthesis of glutamine from glutamate and ammonia. Under nitrogen-rich conditions, GS becomes feedback-inhibited by high intracellular glutamine levels and then binds transcription factors GlnR and TnrA, which control the genes of nitrogen assimilation. While GS-bound TnrA is no longer able to interact with DNA, GlnR–DNA binding is shown to be stimulated by GS complex formation. In this paper we show a new physiological feature of the interaction between glutamine synthetase and TnrA. The transcription factor TnrA inhibits the biosynthetic activity of glutamine synthetase *in vivo* and *in vitro*, while the GlnR protein does not affect the activity of the enzyme.

Structured summary of protein interactions:
GS physically interacts with TnrA by anti bait communoprecipitation (View interaction)
TnrA binds to GS by pull down (View interaction)
GlnK physically interacts with TnrA by anti bait communoprecipitation (View interaction)
GlnK binds to GS by pull down (View interaction)

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1. Introduction

Glutamine synthetase (GS) is a metalloenzyme catalyzing the ATP-dependent synthesis of glutamine from glutamate and ammonia [1,2]. This is a universal reaction for many bacteria and is the predominant mechanism by which inorganic nitrogen is incorporated into cellular metabolites. Since *Bacillus subtilis* does not contain an anabolic glutamate dehydrogenase [3], this is also the only pathway for ammonium assimilation in *B. subtilis*. Glutamine is a key component in nitrogen metabolism and the synthesis as well as activity of GS is strongly regulated by a variety of mechanisms in response to nitrogen availability. The activity of GS is high in cells growing under nitrogen-limiting conditions and is low when cells are growing rapidly with nitrogen excess [4].

GS from *B. subtilis* is not regulated by any known post-translational protein modification [5], but is directly feedback-inhibited by glutamine and several end-products of glutamine metabolism [6]. In addition to glutamine, AMP is the second most effective feedback inhibitor of GS. Glutamine binds to the glutamate substrate site whereas AMP binds at the ATP site [6]. The glutamine biosynthetic activity of *B. subtilis* GS can be assayed *in vitro* and requires Mg⁺ or Mn²⁺ [7]. Mg⁺-dependent biosynthetic reaction is the main physiologically relevant enzymatic activity [1,2] and can be completely inhibited by glutamine or AMP. The Mn²⁺-dependent biosynthetic activity is only partially inhibited by alanine, serine or glycine.

*B. subtilis* GS is a trigger enzyme and has a dual function: as a biosynthetic enzyme for ammonium assimilation and glutamine synthesis and as a regulatory enzyme controlling the activity of transcription factors by direct protein–protein interactions [8,9]. Two transcription factors, termed GlnR and TnrA, control the expression of nitrogen-regulated genes in *B. subtilis* [10,11]. Both of these proteins are members of the MerR family of transcription regulators, contain a helix-turn-helix DNA-binding domain at the N-terminus and bind to the same DNA-consensus sequence [11,12]. Although the sequences of the amino-terminal DNA-binding domains of TnrA and GlnR are highly similar, these proteins have little sequence similarity in their C-terminal signal transduction

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